Bacteriophages in *Helicobacter (Campylobacter) pylori*

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Summary. Bacteriophages in different stages of maturation were found in thin sections of a clinical isolate of *Helicobacter (Campylobacter) pylori*. Mature phage heads measured 70 × 60 nm and the tail at least 120 nm. Lysogeny was maintained during subculture on blood agar for more than 3 months after isolation from a gastric biopsy.

Introduction

Temperate bacteriophages that are capable of both lysogeny and lysis are found in many gram-positive and gram-negative bacteria¹ as well as in mollicutes² and L-forms.³ In clinically relevant species, lysogenic strains contribute to the transfer of resistance, to virulence, and to toxin production by means of transduction.

Marshall and Warren⁴ found gram-negative curved bacilli, later called *Campylobacter pylori*, in the stomach of patients with gastritis and peptic ulceration. Further studies confirmed the strong association of this species with type B gastritis and peptic ulcer disease.⁵ Recently, the name *Helicobacter pylori* was proposed because of many taxonomic differences between "*C.*" *pylori* and the other *Campylobacter* and *Wolinella* species.⁶

We now describe a phage-producing strain of *H. pylori*; this seems to be the first observation of bacteriophages in this species.

Materials and methods

Bacteria

The *H. pylori* strain designated *SchReck* was isolated from a gastric biopsy and identified by rapid urease, catalase and oxidase reactions.

Media

Brain Heart Infusion Broth (Oxoid), containing sheep blood 10% and (L) agar 10 g, vancomycin 6 mg, nalidixic acid 20 mg, amphotericin B 2 mg and triphenyl-tetrazolium chloride 40 mg was used for primary isolation.⁷ Subcultures for electronmicroscopy and gas chromatography were made on Blood Agar Base (Oxoid) with sheep blood 6%.

Antibiotic susceptibility tests were performed by the agar dilution method on Iso-Sensitest Agar (Oxoid) with sheep blood 10%.

Gas chromatography

Colonies were harvested after 3–4 days of microaerophilic incubation on blood agar, and subsequently saponified with NaOH 15% in aqueous methanol. The fatty acids were methylated with acid methanol. The fatty acid methyl esters were extracted with tert-butylmethylether/hexan, and separated on a fused silica capillary column in a Hewlett Packard 5890A gas chromatograph with a flame ionisation detector. The fatty acid profiles were identified by comparison with the data of Lambert *et al.*⁸

Electronmicroscopy

Bacteria were harvested from blood agar and prefixed in glutaraldehyde 2% in cacodylate buffer 0.2 mol/L, followed by osmium tetroxide 1% and uranyl acetate 2%. Dehydrated samples were embedded in glycidether (Epon 812).⁹ Sections were mounted on copper grids, stained for 20 s with lead citrate 4% and examined with an EM 10 (Zeiss) electronmicroscope at an accelerating voltage of 80 kV.

Results

The *H. pylori* strain was isolated from the gastric biopsy of a patient with an acute gastric ulcer. Histological examination showed chronic antral gastritis with intestinal metaplasia and atrophy.

The strain exhibited rapid urease, catalase, and oxidase reactions. Gas chromatography revealed high amounts of fatty acid methyl ester 14:0 and 19:0 delta. Small amounts of 16:0, 18:3-OH and 16:3-OH esters were also present. This pattern is

typical of *H. pylori*.⁸ The strain was susceptible to ampicillin, cefuroxime, gentamicin, tetracycline, co-trimoxazole, ofloxacin and rifampicin (MIC <1 mg/L in each case), but was not inhibited by vancomycin or teicoplanin at a concentration of 1.6 mg/L.

After weekly subculture on antibiotic-free sheep-blood agar over a 3-month period, bacteria were examined by electronmicroscopy. The bacteria exhibited the typical fine structure of gram-negative bacteria, with a smooth outer and inner membrane, separated by a periplasmic space. The organisms also displayed a sheathed flagellum (fig. 1) and an electron-dense submembranous complex, adjacent to the inner membrane and near the flagellar attachment site.

Thin sections also revealed that the *H. pylori* strain spontaneously produced bacteriophages. In each of three independent samples which were subcultured on blood agar and harvested after incubation for 2–4 days, a small number of bacteria released (figs. 1 and 2) or harboured (fig. 3) phage particles with hexagonal heads. Head dimensions were approximately $70 \pm 5 \times 60 \pm 4$ nm after chemical fixation. Up to 100 empty or mature phage particles were found in a thin section of a single cell. Tails of at least 120 nm were discernible in cells with less dense cytoplasm or on extracellular phages (fig. 2).

The number of phages was not sufficient to allow

the structures of isolated particles to be studied in negatively stained preparations, even after ultracentrifugation.

Discussion

Although there have been many bacteriological and histological studies of *H. (C.) pylori* since the first description in 1984 by Marshall and Warren,⁴ bacteriophages have not been described. The failure to detect phages in *H. pylori* by electronmicroscopy,^{10,11} suggests that only a few bacteria in a population harbour the phages or that the prevalence of strains with morphologically detectable phages is low.

The present finding documents the existence of lysogeny in this species, but since the percentage of lysogenic strains is unknown an assessment of the role of phages in *H. pylori* remains conjectural. The fact that not all persons colonised with *H. pylori* develop active and symptomatic gastritis¹² supports the suggestion that strain-specific pathogenicity exists. Such differences in virulence as well as the development of antibiotic resistance during therapy, as described for nitroimidazoles¹³ and for ofloxacin,¹⁴ might be mediated by plasmids or phages.

The search for further lysogenic strains depends on an effective inducing system, and the strain described here will enable the efficacy of induction

Fig. 1. Thin section electronmicrograph of *H. pylori* showing a curved rod and a lysed cell releasing mature phages. Arrows indicate parts of sheathed flagella with dense cores. Bar = 200 nm.

Fig. 2. Thin section electronmicrograph of *H. pylori* showing cell with empty and filled phage heads. Phage tails are discernible in some of the extracellular phages (arrows). Bar = 200 nm.

Fig. 3. Thin section electronmicrograph of *H. pylori* densely packed with bacteriophages. Arrows indicate part of the submembranous structure predominantly found near the attachment site of the flagellum. Bar = 200 nm.

to be tested. However, during the evaluation of a typing system for *C. jejuni* and *C. coli*, Salama *et al.*¹⁵ found that mitomycin and a broth induction method failed to induce any phages in 270 strains of these species.

The role of phages in *H. pylori* requires further investigation.

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